

*Cellular Immunity: Observations on Natural and Acquired
Immunity to Cobra Venom.*

By J. A. GUNN and R. ST. A. HEATHCOTE.

(Communicated by Prof. Sherrington, F.R.S. Received October 16, 1920.)

(From the Pharmacology Laboratory, Oxford.)

Introductory (J. A. Gunn).

While the properties acquired by the serum of an animal as the result of immunisation to a toxin of the bacterial type have been examined with an exhaustive minuteness, little of the now vast literature on immunity has concerned itself with the cellular as opposed to the humoral aspect of immunity. Little, and less that is certain, is known of the changes that immunisation produces in living cells other than the white blood corpuscles. The part played by these cells in immunity processes, so fruitfully studied by Metchnikoff and others, is responsible by itself for an extensive literature which cannot be dealt with here. Accurate information is still wanting in regard to the part played by other living cells in the acquisition and retention of immunity. One reason for this is that investigation has been confined too exclusively to the blood. This has been partly and justifiably due to the diagnostic, therapeutic, and other importance of immune sera; also, perhaps, to the fact that the technique of blood investigation is more easy and generally familiar than the technique necessary to deal with other tissues. Another reason, no doubt, is that there are relatively few toxins which produce true immunity that lend themselves to the kind of investigation adopted in the experiments to be described.

Without attempting the task, here unnecessary, of making a complete survey of the literature on cellular immunity, I wish to state briefly the present state of knowledge in regard to certain factors which have been investigated as explanatory of (a) natural, and (b) acquired immunity.

(a) *Natural Immunity*.—It is now generally believed that natural immunity either to drugs, poisons, or toxins is seldom, if ever, due to the presence of antitoxin in the blood of the immune animal. For example, Calmette and Delearde (1) found that there is no antitoxic substance in the blood of reptiles capable of explaining the relative natural immunity which they possess to venom; and similarly, that the sera of the fowl and tortoise, which resist high doses of abrin, are completely devoid of antitoxic power. Though they did find that the sera of the mongoose and hedgehog

(which both show high immunity to venom) possess antitoxic properties, those properties are slightly developed, and do not accord with the degree of immunity. They therefore concluded that there is no correlation between the naturally refractory state which certain animals possess and the antitoxic power of their sera to the toxins to which they are insensitive.

Camus and Gley (2) found that the serum of the hedgehog possesses no antitoxic action against eel serum, though this animal withstands a dose twenty times greater than the rabbit. Pettit (3) found that the relative immunity of the rat to diphtheria toxin is not due to an antitoxin in the rat's serum.

It may be stated generally, as the result of these and many other observations on a variety of poisons and toxins, that, though isolated cases are known (4) where the serum possesses unusual antagonising powers, natural immunity is not generally to any extent, and probably never completely, due to the presence of antitoxins in the blood.

It has generally been assumed, therefore, from this that natural immunity is due to a specific insensitiveness of the animal cells to the action of the toxin. Positive proof of this assumption has not so often been brought forward. One aspect of it has been carefully examined by Camus and Gley. They found that the red blood corpuscles of the hedgehog (2), as well as of other animals, *e.g.*, the toad, pigeon, and bat (5), which possess a relative natural immunity to eel serum, show an increased resistance to the hæmolytic action of this toxin. Those experiments seemed to establish a relationship between natural immunity and cellular resistance, so far, at least, as the erythrocytes were concerned, but Camus and Gley later found that the red blood corpuscles of the marmot also (6), and of the cat (7), show a high resistance to the hæmolytic action of eel serum, though both animals are extremely sensitive to the toxic action of the serum. The relationship was therefore not constant. They therefore concluded that natural immunity is a complex phenomenon, and that the resistance manifested by one tissue does not imply a similar resistance of the other tissues of the animal towards a particular toxin.

In regard to the natural immunity of the rat to strophanthus (8), I was able to show a clear quantitative relationship between immunity and cellular resistance.

I found that the minimum lethal dose of strophanthus per kilogramme for the rat is thirty times the minimum lethal dose per kilogramme for the rabbit. When the excised hearts of rats and rabbits are artificially perfused with solutions of strophanthus—a procedure which previously removes the serum—it was found that it requires about thirty times as strong a solution

to arrest the rat's heart as is required to arrest the rabbit's heart in the same time.

The conclusion was drawn that the congenital tolerance of the rat to strophanthus is due chiefly, if not entirely, to an insusceptibility of the heart of this animal to the action of strophanthus. This poison was particularly suitable for establishing such a quantitative relationship—if it existed—as arrest of the heart is the primary cause of death in strophanthus poisoning.

Two subsidiary points were established which find a parallel in the experiments to be described in this paper. In the first place, the ratio of 30:1—the concentration necessary to arrest the rat and rabbit heart respectively—held good only for such lower concentrations as arrest the heart slowly; with high concentrations, the ratio was nearer 2:1. The theoretical bearing of these results is discussed in the original paper. In the second place, though the rat's heart showed a specific insusceptibility as compared with the rabbit's heart to the action of strophanthus, comparable to the difference in the lethality of strophanthus for the intact animals, no difference was found between rat's and rabbit's red blood corpuscles in their resistance to strophanthus hæmolysis.

Many years ago, Kanthack (9) made a careful quantitative study of the relationship between the lethality and hæmolytic action of cobra venom for a variety of cold- and warm-blooded animals, and came to the conclusion that "there is no absolute relation between the hæmolytic reaction of the blood of an animal to cobra poison and the resistance of the animal itself to the poison."

We have in these papers a series of observations, made, with different poisons, to determine whether any relationship exists between cellular resistance and natural immunity. From its convenience and familiarity as an experimental cell, it was natural that the red blood corpuscle should be employed in cases where the poison acted on it. It is however important to realise that, alike with eel-serum, cobra venom, and strophanthus, no consistent relation, if any, was found to exist between natural immunity of the animal and resistance of its erythrocyte to hæmolysis. On the other hand, in the case of strophanthus, when the heart was employed, the existence of a quantitative relation between immunity of the animal and resistance of the heart was clearly demonstrable. The significance of this, especially the failure of the red cell as a reliable index of cellular immunity, will be discussed later.

(b) *Acquired Immunity*.—It is of perhaps greater interest to determine whether, when an animal is actively immunised to a toxin, the cells of the

animal become more resistant to the toxin. Relatively few observations seem to have been made on this point, though it might afford an explanation of many problems, for example, why a high degree of immunity may exist with the presence of a feeble antitoxin in the blood, and why immunity should in some cases persist after antitoxin has disappeared from the blood. There can be no doubt that this is a question of fundamental importance which demands a larger share of attention than has so far been bestowed upon it.

Camus and Gley (10) found that the washed red corpuscles of a rabbit immunised to eel serum showed augmented resistance to the hæmolytic action of this serum. No quantitative determinations are given but it is stated that the increased resistance was sometimes very slight.

Kossel (11) stated that the red cells of rabbits immunised to eel serum, and carefully freed from serum, became more resistant to the hæmolytic action and in proportion to the grade of immunity; but he gives no details of the experiments.

Later Camus and Gley (12) found no increased resistance of the red cells in rabbits after prolonged immunisation to eel serum, but in an animal rapidly immunised (by four injections in six days) they found that some of the corpuscles were not hæmolyised even by high concentrations—an effect due, they suppose, to the fact that the less resistant corpuscles have been destroyed.

On the other hand, Cushny (13) found that the blood of a rabbit immunised to 5000 times the minimum lethal dose of ricin still showed agglutination like that of a normal rabbit, only it appeared "even somewhat more sensitive." Jacoby (14) who immunised a goat for eleven months and at intervals tested the resistance of the red cells, found them still agglutinable, when free from serum, even in the highest stages of immunity. He pointed out that the possibility of cellular immunity of the red cells occurring in still higher stages of immunity was not excluded.

Calmette (15) stated clearly, though so far as I can find he did not subsequently publish details of his experiments, that the red blood corpuscles of an animal, immunised to cobra venom and which yielded a very antitoxic and anti-hæmolytic serum, were still perfectly hæmolyisable after being freed from serum, by feeble doses of venom to which had been added a little normal serum heated to 62°.

So far as concerns the red blood corpuscles, therefore, no acquired cellular immunity has been found except to eel serum; and apparently with ricin and cobra venom observers have hesitated to commit themselves to the paradoxical result that the red cells may become actually more sensitive.

In regard to acquired cellular immunity of other tissues, the results are not very convincing. Gley (16) found that the toxicity of eel serum for the rabbit is ten times greater when injected into the cerebro-spinal fluid than when injected intravenously. When a rabbit was immunised the minimum lethal dose by cerebro-spinal injection was practically unaltered, and he therefore concluded that the central nervous system does not acquire immunity to eel serum, or at least only a very feeble immunity.

Gley (17) came to the same conclusion with torpedo serum, namely, that there was no cellular immunity as far as the central nervous system was concerned.

These experiments were of the same type as those of Roux and Borrel (18) who found that rabbits immunised against tetanus are as easily as before made tetanic by injections into the brain itself. Such experiments are ill adapted to prove the point at issue, because, though relatively small amounts of toxin are injected into the brain or cerebro-spinal fluid, they are injected in high concentration compared to that which arrives at the central nervous system when the toxin is diffused through the blood.

Gley and Pachon (19) prosecuted further researches on this problem to determine whether there was any cellular immunity developed in the heart when an animal is immunised to toxic sera. Their experiments were inconclusive. Indeed the toxins employed were not suitable for deciding the point. It required 1 to 4 per cent. of eel serum and 10 to 20 per cent. of torpedo serum to produce even doubtful effects on the heart. Cushny and Gunn (20) have shown more definite effects with lower concentrations of horse serum, so that it is doubtful if the serum of the eel or torpedo has any specific toxic action on the heart.

So far as acquired immunity is concerned these observations leave the question still undecided. According to Camus and Gley and Kossel, in an animal immunised to eel serum the red cells, freed from antitoxin, may show an increased resistance to the hæmolytic action of the serum, but a similar effect was not found by Cushny or Jacoby with ricin or by Calmette with cobra venom, and no convincing proof was forthcoming of an increased cellular immunity of the heart or central nervous system occurring in the process of immunity.

It was my intention to proceed, after completing the experiments described with strophanthus, to determine by similar methods whether when an animal is immunised to a particular toxin, the tissues of the animal freed from the serum acquire an increased insusceptibility to the action of the toxin. For this purpose it was necessary to employ a toxin of the bacterial type which produces genuine immunity with antibodies in the serum. Of such toxins

among the most convenient to work with, for well-known reasons, are either the snake venoms or one of the vegetable toxins, such as ricin.

I have at various times begun such experiments with such samples of snake venom as I could procure, but have been unable to complete them from want of a sufficient amount of a sufficiently active venom. All the experiments have, of course, to be completed with the same sample of venom. I also tried ricin, which was from one point of view more suitable, because in contradistinction to what is true of snake venoms it is extremely easy to immunise rabbits to ricin. But unfortunately I found that ricin produced no effect on the isolated heart or intestine of the rabbit, even in a concentration of 1 in 5000, although the minimum lethal dose of it for this animal was 0.05 mgrm. per kilogramme. It was clearly unsuitable for this kind of investigation. I found, however, that the red blood corpuscles of an animal immunised to ricin are *more* easily agglutinated than are the red blood corpuscles of a normal rabbit.

Cushny, as has been pointed out, had found that the blood of a rabbit immunised to 5000 times the minimum lethal dose of ricin still showed agglutination like that of a normal rabbit, only it appeared even somewhat more sensitive. As a matter of fact the increased sensitiveness is well beyond experimental error. For example, I found by parallel experiments that the washed corpuscles of a rabbit immunised to twenty minimum lethal doses of ricin were agglutinated by 0.0005 per cent. of ricin as compared with 0.002 per cent. required to agglutinate washed corpuscles of a normal rabbit. Those observations suggested to me a doubt as to whether the red cells could be a reliable index of acquired cellular immunity, presuming that such a condition occurred. As has been pointed out above, they have certainly not been found to be so in natural immunity, and *a priori* it is less likely that they should be so in acquired immunity for these reasons. The mammalian red cell is a cell that has lost its nucleus. It is probably not a living cell in the ordinary sense at all but a mere box with a transitory existence. It is difficult to imagine in what way the *formed* red cell could develop an increased resistance in the process of immunisation. It is true that a more resistant cell might be manufactured in the bone marrow and so be distributed into the blood. But on the whole it seemed unwise to employ a cell that is, both histologically and in regard to its passing existence, different from most cells of the body as an index of what occurs, in the process of immunisation, to the cells of the body generally.

I am not suggesting—indeed the experiments of Camus and Gley and Kossel point otherwise—that at no period in the course of immunisation will an increased resistance be found in the red cells if the other cells of the body

acquire it. But the experiments to be described, I think, render the red cells still further suspect as a test of cellular immunity. The peculiar character of the red cell has not been sufficiently realized and, it may be, has been the means of preventing work on cellular immunity, for though it is the test object that would first suggest itself, it is the most misleading guide to the condition of the cells generally.

In 1912, as a preparation for the scheme of experiment now undertaken, I investigated in greater detail than had hitherto been done the action of cobra venom on isolated mammalian tissues. I came to the conclusion (21) that cobra venom contains some substance having an action closely approximating to a sympathomimetic action. Cushny and Yagi later (22), working with a different sample of venom, obtained results differing in certain particulars, especially in the fact that they obtained no inhibitory action on smooth muscle and no stimulation of secreting glands such as I had obtained; and they therefore came to the conclusion that cobra venom does not contain any substance of sympathomimetic action. In regard to a difference of results of this nature, it is well to keep in mind that snake venoms are, so far as is known, substances of complex nature and presumed to contain a variety of toxins of varying stability. Complete concordance of results in regard to primary action cannot, therefore, be expected with different samples of venom, collected in different ways, and kept under different conditions and for different times. It is, however, interesting to find that Abel and Macht (23) have discovered in the skin secretion of the toad two substances (*a*) a substance having an action like digitalis, and (*b*) adrenaline. Cushny and Yagi found that cobra venom acted rather like digitalis, while I found that it acted rather like adrenaline. Perhaps the question as to whether both may not occur in cobra venom, as in toad poison, ought to be left open. The question ought to be decided by determining the action of a perfectly fresh venom and by examining the glands for the chromaphil reaction. In any case the venom I have now used resembles more nearly that employed by Cushny and Yagi. Especially, this sample of venom caused a marked rise of tone in the isolated rabbit's intestine; but the addition of a minute trace of adrenaline (1 in 30,000,000) prevented this rise of tone even with high concentrations of venom and produced an effect exactly resembling that previously found by me.

Observations on Natural and Acquired Immunity to Cobra Venom. (Gunn and Heathcote.)

The Congenital Immunity of the Cat to Cobra Venom.

It has long been known that animals show different degrees of susceptibility to the toxic action of venoms, and especially that the cat shows a high degree of resistance as compared with rodents. Thus Fraser (24) in 1896 showed that the minimum lethal dose of cobra venom for the cat is thirty times that for the rabbit, and Fraser and Gunn (25) that the minimum lethal dose of *Sapedon haemachates*—another colubrine venom—is for the cat fifteen times that for the rabbit. This comparative tolerance of the cat is most marked in the case of colubrine venoms, but it is also true to a certain extent of viper venoms, for Fraser and Gunn (26) found that the ratio of lethality for the cat and rabbit in the case of *Echis* venom was 9 : 1.

Minimum Lethal Dose for Rabbit and Cat.

The minimum lethal dose of the venom we used was found to be for the rabbit 0·0012 grm. per kilogramme and for the cat 0·025 gramme per kilogramme. Calculated per kilogramme, therefore, the minimum dose required to kill the cat is twenty times greater than that required to kill the rabbit. As this relation is similar to that found by Fraser and others, it is not necessary to give the experiments in detail.

The following experiments were made to ascertain whether or not, corresponding to this relative natural immunity of the intact animal, the tissues of the cat are less susceptible to the action of the venom than are the tissues of the rabbit. With this end in view, experiments were made on the heart, intestine, and red blood-corpuscles.

(a) *Heart.*

For perfusing the heart and for recording the flow through the coronary vessels, the heart-perfusion apparatus (27) and syphon-recorder (28), described by one of us were employed. As the perfusing solution, Locke's solution without glucose was used. The hearts were perfused for at least twenty minutes before the venom solution was tried, so that all the serum was removed from the heart first.

Cobra venom, if in sufficient concentration, kills the heart by arresting it in the systolic position. The preliminary effects are somewhat variable, there being sometimes a primary enfeeblement of systolic contraction, occasionally a small and short-lasting augmentation or acceleration.

Table I.—Comparison of the Action of Cobra Venom on the Perfused Heart of the Rabbit and Cat.

Strength of solution.	Rabbit.	Cat.
1 in 5,000	Systolic arrest in 8 minutes	Systolic arrest in 7 minutes.
" 10,000	" " 8 "	—
" 20,000	" " 8 "	—
" 50,000	" " 10 "	Systolic arrest in 11 minutes.
" 100,000	" " 15 "	" " 17 "
" 200,000	" " 13 "	" " 31 "
" 400,000	" " 15 "	" " 34 "
" 600,000	" " 20 "	Not arrested in 80 minutes.
" 800,000	" " 34 "	" " 80 "
" 1,000,000	—	" " 105 "
" 2,000,000	Systolic arrest in 73 minutes	—

This table shows clearly that the heart of the cat is much less susceptible than that of the rabbit to the action of the venom. This does not appear with high concentrations, *e.g.*, there is no appreciable quantitative difference in the action on the rabbit and cat heart in concentrations ranging from 1 in 5000 to 1 in 100,000. With lower concentrations, there is an unmistakable difference in the susceptibility of the hearts of the two species of animals, whether this be judged from the time required to produce a given effect, or from the minimum concentration required to kill the heart in systole. Thus, 1 in 200,000 arrests the heart of the cat in 31 minutes, whereas half that strength (1 in 400,000) arrests the rabbit's heart in half the time. Or a strength of 1 in 200,000 produces practically the same effect on the cat's heart as one of 1 in 800,000 on the rabbit's. Judged from this standpoint, the immunity of the cat's heart is to that of the rabbit's as 4:1. Further, a solution of 1 in 2,000,000 arrests the rabbit's heart in 73 minutes, whereas a solution of 1 in 800,000 does not arrest the cat's heart in 80 minutes.

In figs. 1 and 2, the effects of 1 in 600,000 are shown on the hearts of the rabbit and cat. The rabbit's heart is completely arrested in 20 minutes, whereas the cat's heart is not markedly affected in 70 minutes.

It is clear, therefore, that the cat's heart, as compared with the rabbit's, possesses in itself a tolerance to cobra venom. A larger number of experiments would be required to give an exact numerical ratio to the difference in susceptibility, if, indeed, it would even then be possible in such experiments to give exactly such a ratio. For the present purpose, it is sufficient to establish the fact that the difference is pronounced, and that, judged from an arbitrary end-point, it requires four times as strong a solution to produce a given effect on the cat's heart as is required to produce that effect on the rabbit's.

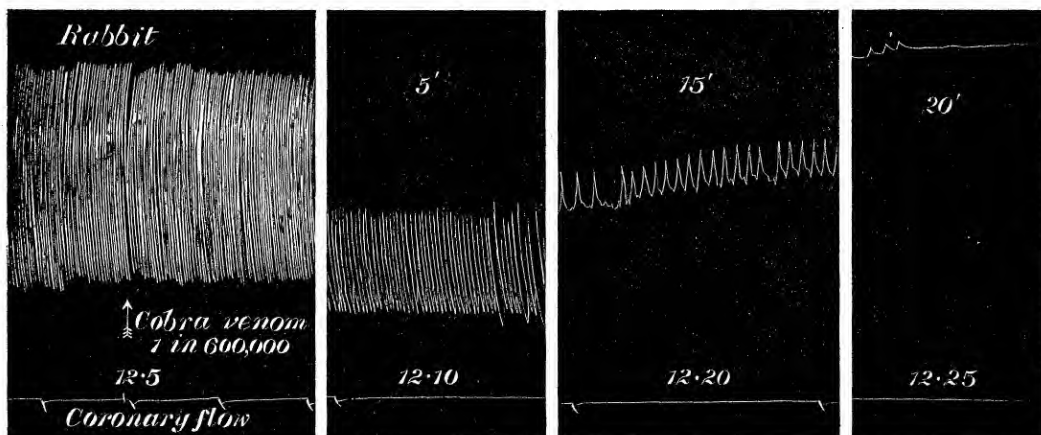


FIG. 1.—Isolated rabbit heart perfused with cobra venom, 1 in 600,000.

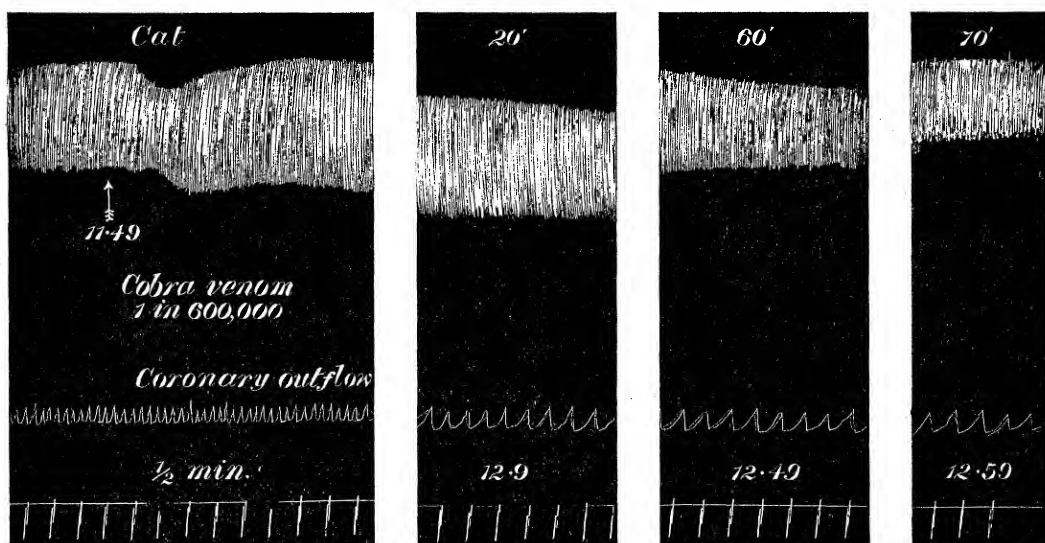


FIG. 2.—Isolated cat heart perfused with cobra venom, 1 in 600,000. Showing that whereas the rabbit heart is completely arrested in systole in 20 minutes, the cat heart is not markedly affected in 70 minutes by the same strength of solution.

(b) *Coronary Blood-vessels.*

When either the rabbit's or the cat's heart is perfused with solutions of cobra venom, the flow through the vessels is markedly diminished as soon as the ventricle goes into systolic contraction. This effect occurs with other substances, which produce rigor of the heart muscle. The flow is stopped by compression and indirect occlusion of the vessels, as the result of the contracture of the heart muscle surrounding them.

The chief point of interest lies in the effect of the venom on the coronary flow prior to the onset of rigor of the heart. In the rabbit, the flow always diminishes from the beginning, independently of the alteration in the heart beat. The effect is therefore no doubt a direct action on the arterial wall, similar to that which occurs with other types of smooth muscle, and which will be dealt with in the next section. In the cat, on the other hand, there is generally a preliminary increase in the coronary flow, sometimes to the extent of over 50 per cent., which may last for several minutes. This, again, is independent of changes in the heart's contractions, and is therefore probably due to the venom causing a preliminary dilatation of the coronary arteries. The effect is not constant, and in its nature and occurrence resembles the effect produced by the venom on the smooth muscle of the intestine, which in the cat is sometimes relaxed by the venom.

The conclusions we draw from these experiments are (1) that the heart muscle of the cat is much less susceptible to the toxic action of the venom than is the heart muscle of the rabbit, and (2) that there is a qualitative difference in the reaction of the coronary arteries of the two species of animals, those of the cat being usually at first relaxed by the venom, those of the rabbit always contracted from the beginning.

(c) *Intestine.*

A large number of experiments were performed to compare the effects of cobra venom on the intestine of the rabbit and of the cat. These experiments were performed by the now familiar method of Magnus, sections of the small intestine, about 2 cm. in length, being suspended in a bath of oxygenated Locke's solution at 37° C. The bath contained 50 c.c., and the venom was added in solution in known amounts, so that the resulting concentration of it could easily be calculated.

For the purpose of an investigation like the present, the intestine does not give such reliable quantitative results as the perfused heart. When the heart is perfused, the venom acts uniformly throughout the substance of the heart muscle, whereas when the venom is added to the solution surrounding the suspended intestine, the action to begin with is a superficial action. Substances which act on the nerve-endings, like pilocarpine or adrenaline, exert their actions almost instantaneously on the isolated intestine when the effects are recorded in the manner here adopted. With substances that act on the muscle, like cobra venom, which is also probably slowly diffusible, there is a definite delay in the production of the full effect of a certain concentration on the isolated (non-perfused) gut, a delay due to the fact that the venom reaches superficial muscle fibres before deeper ones.

This fact is mentioned only because time effects and possibly even quantitative effects of the venom cannot be gauged with the same accuracy on the intestinal muscle as on the perfused heart. There is also more irregularity in the quantitative results on different segments of intestine than one obtains in the perfused heart. This is true of other substances, *e.g.*, adrenaline, as well as of cobra venom.

The following summary, however, shows that, in spite of these drawbacks, the effects of cobra venom on the intestine of the cat differ sufficiently, and sufficiently uniformly from its effects on the intestine of the rabbit to allow of conclusions being drawn from the experiments.

With the rabbit's intestine, a solution of 1 in 10,000, 1 in 20,000, and 1 in 25,000 caused tonic contraction of the gut with arrest of the movements in a few minutes, and 1 in 50,000 stopped the movements in the same fashion in half an hour. With the cat's intestine, a solution of 1 in 5,000 caused a slight preliminary rise of tone, which gradually passed off and the movements ceased in a position of relaxation in 25 minutes. 1 in 10,000 caused relaxation of the gut, sometimes marked, but did not arrest the movements in half an hour; 1 in 20,000, 1 in 25,000, and 1 in 50,000 caused slight relaxation, but had very little effect on the amplitude of the segmentation movements, which were still vigorous at the end of half an hour. The experiments with each concentration were repeated twice.

In figs. 3 and 4 is shown a comparison of the differences on the rabbit and cat intestine of cobra venom 1 in 10,000.

These experiments therefore show that there is both a quantitative and a qualitative difference in the action of the venom on the intestine of the rabbit as compared with that of the cat. The quantitative difference consists in the fact that much higher concentrations are required to kill the intestine of the cat than to kill that of the rabbit, and the qualitative difference in the fact that the type of effect differs somewhat in the two animals. In the rabbit the venom always causes arrest of the intestinal movements in systole, while in the cat the venom usually produces increased relaxation.

Red Blood Corpuscles.

Experiments were made on washed red blood corpuscles of the cat and rabbit. A 4 per cent. suspension, twice centrifuged and washed, was used in each case. The saline solution used was 0.9 per cent. sodium chloride in distilled water. The venom solution was made by dissolving the venom in the saline solution. This venom solution was divided into two parts, one of which was used for the experiment on rabbit's corpuscles, the other for that on cat's corpuscles. This obviated experimental errors in weighing and differences in

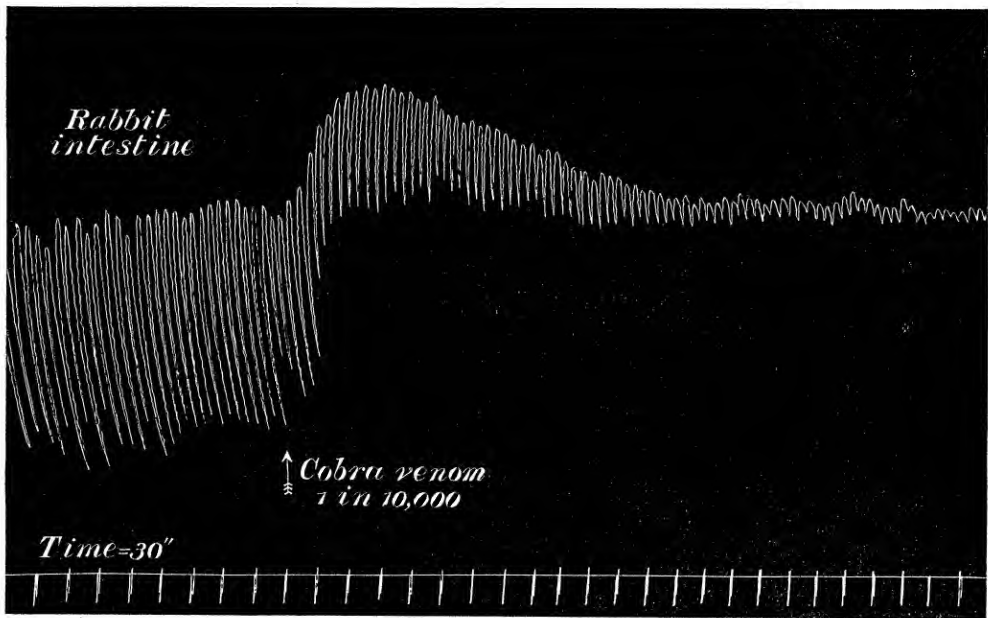


FIG. 3.—Isolated rabbit intestine.

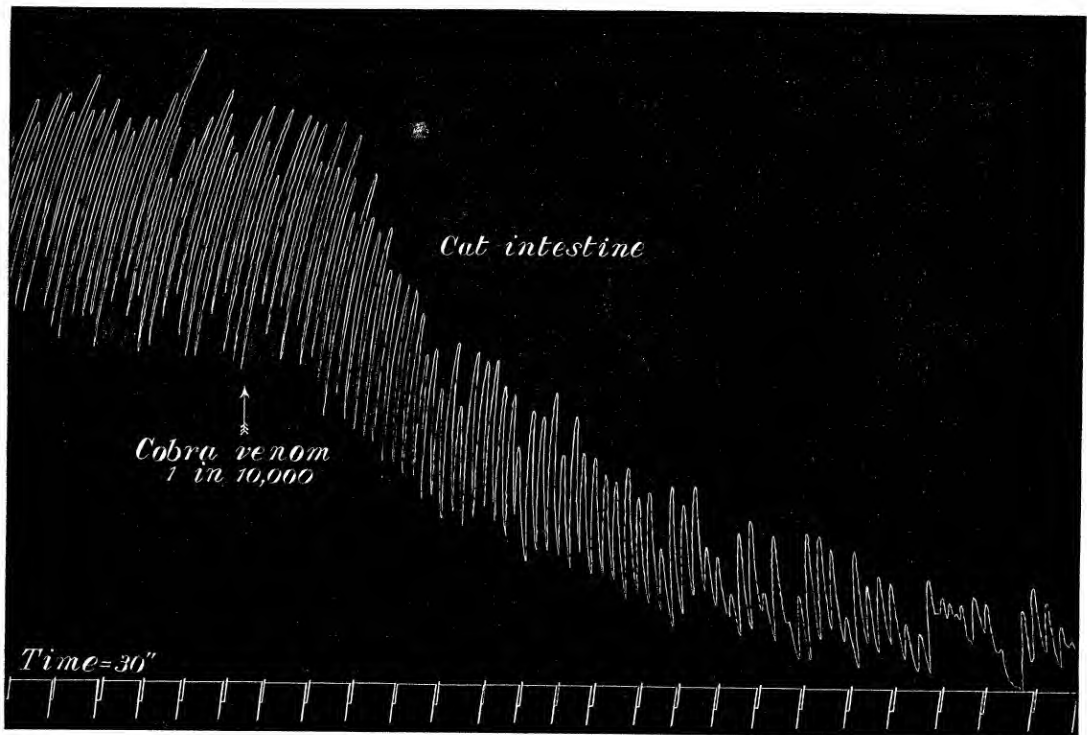


FIG. 4.—Isolated cat intestine. Showing difference in the effects of cobra venom, 1 in 10,000.

the composition of the venom. The times of centrifuging and keeping the blood of the two animals were exactly the same, so that the experiments were as nearly as possible paralleled.

The following Table gives a skeleton of the experiments performed to determine the minimum hæmolytic dose of the venom for rabbit's and cat's corpuscles respectively :—

Table II.—Scheme of Hæmolytic Experiments.

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
	C.V. 0·1 per cent.					C.V. 0·01 per cent.				
Blood suspension, c.c. ...	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25	0·25
Cobra venom, c.c.	0·25	0·2	0·15	0·1	0·05	0·25	0·2	0·15	0·1	0
Saline solution, c.c.	0	0·05	0·1	0·15	0·2	0	0·05	0·1	0·15	0·25
Concentration of venom per cent.	0·05	0·04	0·033	0·02	0·01	0·005	0·004	0·0033	0·002	0

The tubes, therefore, each contained the same amount of corpuscles (as far as can be judged without actually counting them), the same concentration of sodium chloride, and the same amount of total solution, but differed only in their venom-content.

Two parallel experiments were done in this way with rabbit's and cat's corpuscles. The tubes were incubated at 35° C. In 3½ hours the cat's corpuscles were hæmolysed completely in tubes 1, 2, 3, and 4, and just not completely in 5. The rabbit's corpuscles were also completely hæmolysed in tubes 1, 2, 3, and 4 and there was a trace of hæmolysis in 5. There was no trace of hæmolysis in any of the other tubes. The tubes were re-shaken, allowed to stand at room temperature overnight and read 15 hours later. The cat's corpuscles were then found to be completely hæmolysed in the first six tubes and the rabbit's in the first five. Otherwise stated, in 3½ hours cat's corpuscles are completely hæmolysed by a concentration of 0·02 per cent. and almost completely hæmolysed by 0·01 per cent. Rabbit's corpuscles are completely hæmolysed by 0·02 per cent. and slightly hæmolysed by 0·01 per cent. In 18½ hours cat's corpuscles are completely hæmolysed by 0·005 per cent. and rabbit's corpuscles by 0·01 per cent., under the same temperature conditions. There is, therefore, only a slight difference between the susceptibility of the cat's and rabbit's corpuscles to the hæmolytic action of the cobra venom, the cat's corpuscles being hæmolysed by slightly *lower* concentrations.

The conclusion to be drawn from these experiments is that, though the

cat possesses, as compared with the rabbit, a high degree of congenital immunity to cobra venom, the red blood corpuscles of the cat are less resistant to the hæmolytic action of the venom than are the red blood corpuscles of the rabbit.

Acquired Immunity to Cobra Venom.

The object of these experiments was to determine whether, when an animal is actively immunised to cobra venom, the tissues of the animal, free from the serum, acquire increased resistance to the venom. For this purpose we compared quantitatively the effects of the venom on the heart, intestine and blood corpuscles of an immunised animal with its effects on the same organs of normal unimmunised animals.

The difficulty of immunising rabbits to cobra venom is well known and it is not necessary for the purposes of this paper to deal with the question. It is sufficient to state that, of twelve rabbits, which we began to immunise in February, 1920, only one survived immunisation to the degree of tolerating 10 minimum lethal doses. This rabbit received the injections intravenously into the marginal veins of the ear. The doses were increased very gradually. It weighed 1420 grm. on February 7, 1920, the day on which it received its first venom injection, and on June 6, 1920, when it received its last injection of 10 M.L.D., it weighed 2580 grm. This last injection caused a fall of weight on the following day to 2450 grm. but the animal recovered its normal weight by June 10 when it was killed and its isolated organs used for experiments.

The following experiments were performed :—

(a) *Heart.*

In Table I was shown the effect of the venom we were employing on the isolated hearts of normal rabbits. In these experiments perfusion with Locke's solution was carried on for at least 20 minutes to remove all the serum. The same procedure and technique was employed for the heart of the immunised rabbit.

In the following Table III the heart of the immunised rabbit is compared with the hearts of unimmunised rabbits in respect to their susceptibility to the toxic action of the venom.

The heart of the immunised rabbit was first perfused with a concentration of venom of 1 in 400,000 for 45 minutes. In this time only a slight slowing and rise in tone was produced. The concentration of venom perfused was then doubled and arrest in systole was produced in 20 minutes after turning on the new solution.

When these effects are compared with the effects of the venom on the

heart of unimmunised rabbits, it is clear that as the result of immunisation, the heart becomes much less sensitive to the action of the venom, quite apart from any antitoxic action of the serum, because in these experiments the serum is previously washed out by perfusion. A concentration of 1 in 400,000 has less effect on the excised heart of an immunised rabbit than has a solution of 1 in 800,000 on the excised heart of an unimmunised rabbit. Figs. 5 and 6 show this difference in action. In fig. 5 is shown the perfusion of a normal heart with 1 in 800,000, and in fig. 6 the perfusion of the heart of the immunised animal with 1 in 400,000. It is clear that, though in the latter case the concentration perfused is doubled, the toxic effect produced is far less.

In short, the heart of a rabbit immunised to 10 M.L.D. was able to withstand with only slight changes in its action, twice the concentration of venom that sufficed to arrest the heart of an unimmunised animal.

Further, when the immunised heart was subsequently perfused with a concentration of 1 in 200,000, it was only arrested in systole in 20 minutes, corresponding to the time of arrest produced by 1 in 600,000 in the normal heart, in spite of the fact that the former had been previously perfused for 45 minutes with 1 in 400,000.

Though these experiments are insufficient to enable an exact numerical ratio to the quantitative effects of the venom upon the immunised and non-immunised heart to be given, it is justifiable to state that in a rabbit immunised to 10 M.L.D., the heart is at least three times less sensitive to the action of the venom than is the heart of an unimmunised rabbit.

Table III.—Comparison of the Action of Cobra Venom on the Normal and Immunised Heart.

Strength.	Normal rabbit.	Immunised rabbit.
1 in 200,000	Systolic arrest in 13 minutes	Systolic arrest in 20 minutes.
" 400,000	" " 15 "	Very slight effects in 45 minutes.
" 600,000	" " 20 "	—
" 800,000	" " 34 "	—

(b) *Coronary Flow.*

As has been stated in a preceding section (p. 90), when the normal rabbit's heart is perfused with cobra venom, the rate of flow through the coronary vessels is diminished from the first. When the heart of the immunised rabbit was perfused with 1 in 400,000, there occurred a preliminary increase of flow lasting 7 minutes and amounting at its

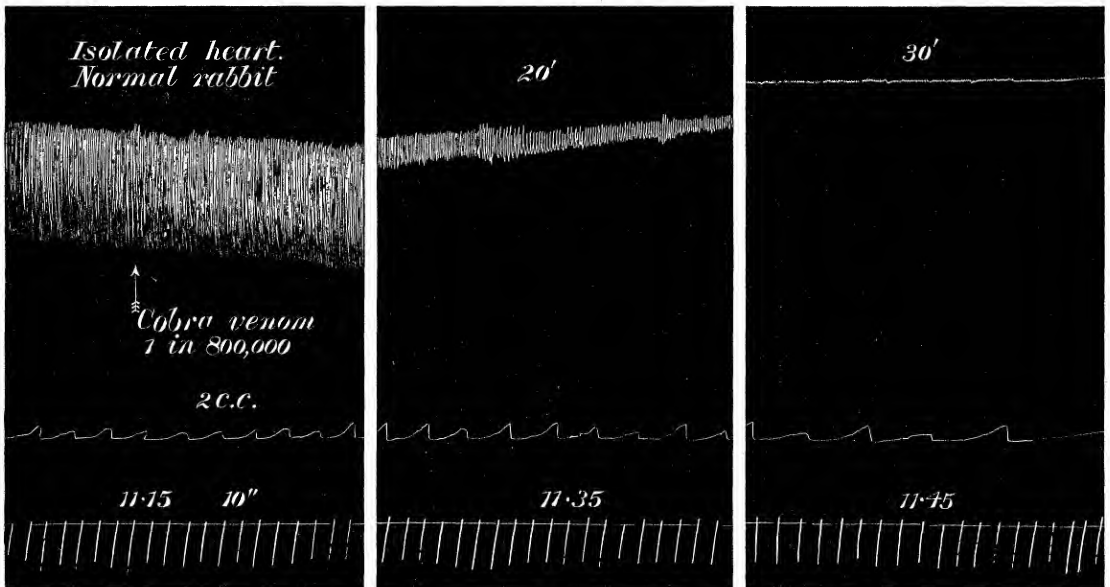


FIG. 5.—Isolated heart of normal rabbit perfused with cobra venom, 1 in 800,000.

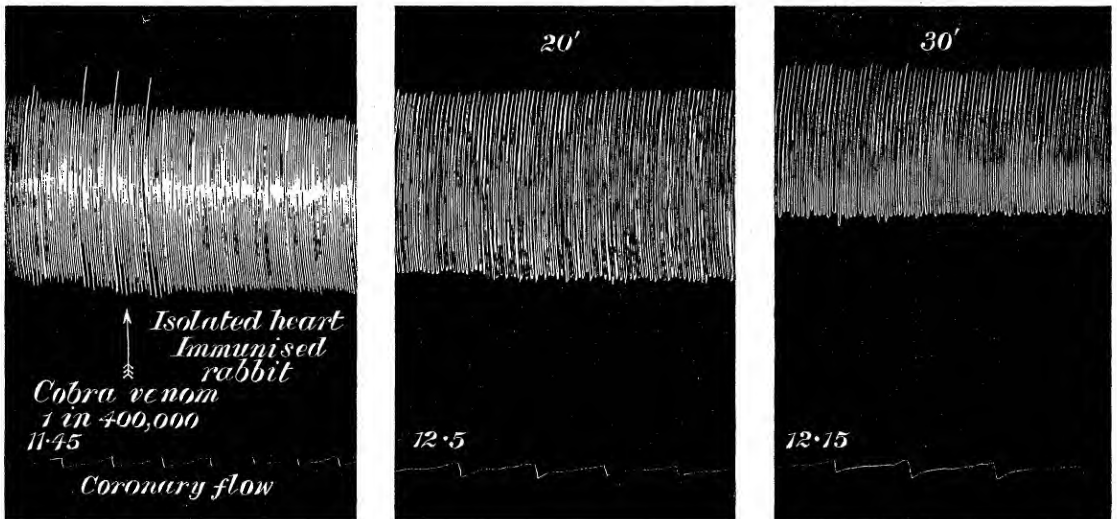


FIG. 6.—Isolated heart of immunised rabbit perfused with cobra venom, 1 in 400,000. Showing that the toxic effect on the heart of the normal rabbit is far greater than that produced, by twice as high a concentration, on the heart of the immunised rabbit.

maximum to a 50 per cent. increase 3 minutes after the venom perfusion began. It would be unwise to lay stress upon a single experiment of this kind, but it is none the less suggestive that the preliminary increase of coronary flow occurs in the cat's heart, which shows a relative natural

immunity, as if the type of effect in the acquired immunity was approximating to that of congenital immunity.

(c) *Intestine.*

In order to get rid of effects due to the presence of antitoxin, it was necessary to wash out the serum from the gut previously to trying the action of the venom. For these experiments, therefore, the gut was washed out *in situ* by perfusion with warm Locke's solution by means of a cannula tied into the aorta, and connected with an elevated reservoir containing the perfusing solution. This was done both in the case of the normal and immunised animal, in order that the intestine should go through the same manipulations in each case. Segments of the gut were afterwards cut out and used for experiment in the ordinary way, and a large number of experiments could in this case be done with the gut of one animal. It was found in the case of the intestine as in the case of the heart that the muscle of the immunised animal, free from serum, was less sensitive to the toxic action of the venom than that of the normal animal. In figs. 7 and 8 is shown a comparison of the effect of 1 in 10,000 upon the normal gut with that of 1 in 5,000 upon the gut of the immunised animal. Though in the latter case the concentration was twice as great, the toxic effect is conspicuously less.

It follows from these experiments that, when an animal is immunised to cobra venom, the tissues of the gut acquire an increased resistance to the toxic action of the venom, apart from the protective action of the antitoxin serum.

(d) *Red Blood Corpuscles.*

The method of experiment and technique were the same as in the experiments previously described (p. 94).

The venom solution was again divided and used for both experiments, so that the corpuscles of the normal rabbit and of the immunised rabbit were subjected to exactly the same concentration of venom. The corpuscles were thrice washed, and the saline solution used was 0·85 per cent. sodium chloride in distilled water.

In 3½ hours the following changes had taken place:—(a) Normal corpuscles. Complete hæmolysis by venom in concentrations down to 0·01 per cent., partial hæmolysis with 0·005 per cent., and no hæmolysis with less concentrations. (b) Corpuscles of immunised animals. Complete hæmolysis by venom in concentrations down to 0·0033 per cent., partial hæmolysis with 0·002 per cent., and no hæmolysis with less concentrations.

In other words, the corpuscles of the immunised animal are more easily

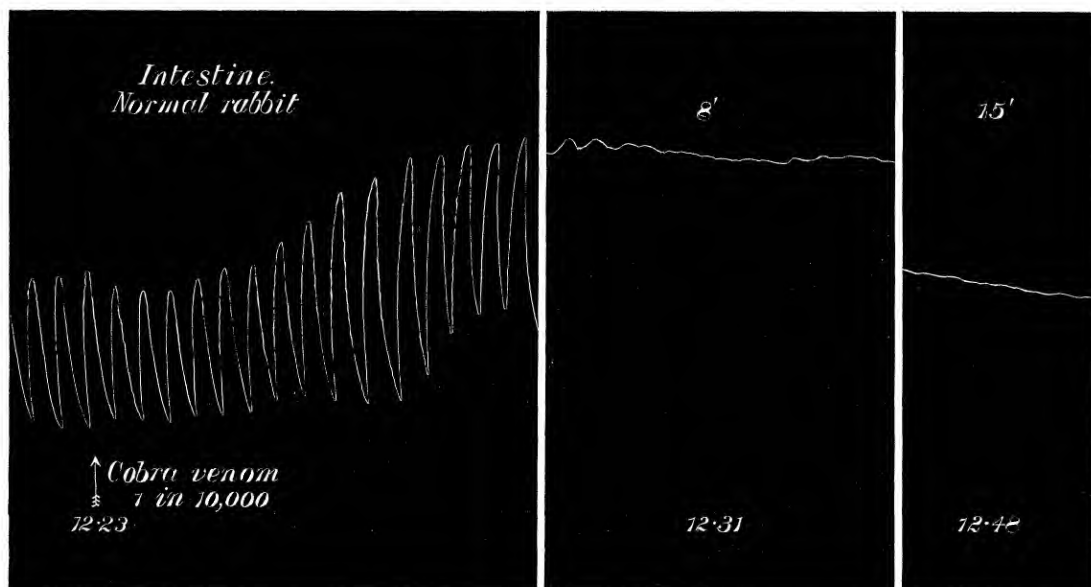


FIG. 7.—Isolated intestine of the normal rabbit.

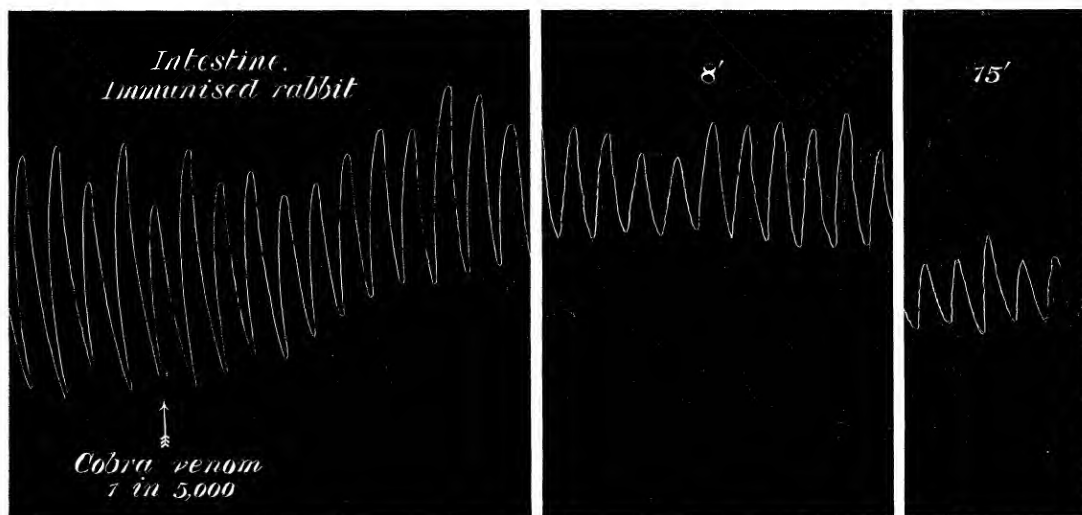


FIG. 8.—Isolated intestine of immunised rabbit. Showing that the toxic effect of 1 in 5,000 on the latter is less than that of 10,000 on the former.

hæmolyse than the corpuscles of the unimmunised animal. In the case of the rabbit immunised to 10 M.L.D., the corpuscles were hæmolyse by about one-third of the hæmolytic dose for the normal animal.

These results confirm what was found for the agglutination of red cells

of immunised and non-immunised animals by ricin. The result seems to be one of considerable importance, because it shows that whereas the heart and intestine become less susceptible to cobra venom in the process of immunisation the red blood corpuscles actually become more sensitive. The most probable reason for this difference is the one suggested in the introduction to this paper, namely, that the red cells, having lost their nuclei, and being no longer living cells in the ordinary sense of the word, cannot respond to the action of repeated doses of the venom in the same way as nucleated cells.

Summary and Conclusions.

(a) *Natural Immunity*.—The minimum lethal dose of cobra venom for the cat is twenty times that for the rabbit (by subcutaneous injection per kilogramme). When the excised hearts of rabbits and cats are artificially perfused with Locke's solution so as to remove the serum, it is found that it requires at least four times as strong a solution of venom to arrest the cat's heart as is required to arrest the rabbit's heart. Similarly the isolated intestine of the cat can withstand the toxic action of higher concentration of venom than can the isolated intestine of the rabbit. The natural immunity of the cat to cobra venom is therefore, in part at least, due to a cellular immunity of the tissues of this animal. No such cellular immunity is displayed by the red-blood corpuscles, those of the cat being actually more sensitive than those of the rabbit to the hæmolytic action of the venom.

(b) *Acquired Immunity*.—When a rabbit is immunised to cobra venom, the isolated heart and intestine, perfused with Locke's solution so as to remove the serum, withstand higher concentrations of venom than the heart or intestine of a normal unimmunised rabbit. In the process of acquired immunity, therefore, some of the tissues at all events develop a cellular immunity, apart from the antitoxin circulating in the serum.

In the rabbit immunised to cobra venom the red blood corpuscles, freed from serum, become, on the other hand, more sensitive to the hæmolytic action of the venom. At the stage of immunity which has been examined, they display no cellular immunity. When a rabbit is immunised to ricin, the red cells also become more sensitive to the agglutinating action of this toxin.

It is clear, therefore, that neither in natural nor in acquired immunity can the red blood corpuscles be taken as a reliable index of cellular immunity. This difference between them and the other tissues examined is, it is suggested, due to the fact that the structure and life history of the red blood corpuscles is different from that of the other cells of the body.

While other results may occur with other toxins, and possibly even with

the same toxins at different stages of immunity, the fact that a cellular immunity of such tissues as the muscle of the heart and intestine can be produced is regarded as proved.

The extent to which different tissues acquire and retain a cellular immunity, beyond and independent of the presence of antitoxin in the serum, and the universality or otherwise of the occurrence of cellular immunity with different toxins requires further investigation.

REFERENCES.

- (1) Calmette et Delearde, 'Ann. Inst. Pasteur,' vol. 10, p. 675 (1895).
 - (2) Camus et Gley, 'C. R. Acad. Sci.,' vol. 127, p. 330 (1898).
 - (3) Petit, 'Ann. Inst. Pasteur,' vol. 28, p. 663 (1914).
 - (4) Phisalix et Bertrand, 'C. R. Soc. Biol.,' pp. 639 and 751 (1895).
 - (5) Camus et Gley, 'Ann. Inst. Pasteur,' vol. 13, p. 779 (1899).
 - (6) Camus et Gley, 'C. R. Acad. Sci.,' vol. 140, p. 1717 (1905).
 - (7) Camus et Gley, 'C. R. Soc. Biol.,' vol. 71, p. 158 (1911).
 - (8) Gunn, 'Journ. of Pharmacol. and Exp. Therap.,' vol. 4, p. 225 (1913).
 - (9) Kanthack, 'Sci. Mem. Medical Officers Army in India,' vol. 4, p. 33 (1898).
 - (10) Camus et Gley, 'Arch. Internat. de Pharm. et de Therap.,' vol. 5, p. 247 (1898).
 - (11) Kossel, 'Berl. Klin. Woch.,' p. 152 (1898).
 - (12) Camus et Gley, 'Ann. Inst. Pasteur,' vol. 13, p. 779 (1899).
 - (13) Cushny, 'Arch. f. Exp. Path. u. Pharm.,' vol. 41, p. 439 (1898).
 - (14) Jacoby, 'Beitr. z. Chem. Phys. u. Path.,' vol. 2, p. 535 (1902).
 - (15) Calmette, 'C. R. Acad. Sci.,' vol. 134, p. 1446 (1902).
 - (16) Gley, 'C. R. Acad. Sci.,' vol. 145, p. 1210 (1907).
 - (17) Gley, 'C. R. Assoc. Franç. pour l'Avanc. des Sci.,' vol. 37, p. 608 (1908).
 - (18) Roux et Borrel, 'Ann. Inst. Pasteur,' vol. 12, p. 225 (1898).
 - (19) Gley et Pachon, 'C. R. Acad. Sci.,' vol. 147, p. 813 (1909).
 - (20) Cushny and Gunn, 'Journ. of Pharm. and Exp. Ther.,' vol. 5, p. 1 (1913).
 - (21) Gunn, 'Quart. Journ. Exp. Phys.,' vol. 5, p. 67 (1912).
 - (22) Cushny and Yagi, 'Phil. Trans.,' B, vol. 208, p. 1 (1918).
 - (23) Abel and Macht, 'Journ. of Pharm. and Exp. Ther.,' vol. 3, p. 319 (1912).
 - (24) Fraser, 'Roy. Soc. Proc., Edin.,' vol. 20, p. 454 (1896).
 - (25) Fraser and Gunn, 'Phil. Trans.,' B, vol. 200, p. 241 (1909).
 - (26) Fraser and Gunn, *ibid.*, vol. 202, p. 1 (1911).
 - (27) Gunn, 'Journ of Physiol.,' vol. 46, p. 506 (1913).
 - (28) Gunn, 'Journ. of Physiol.,' vol. 47, p. iii (1913).
-